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14. ABSTRACT

This project tests the hypothesis that blocking neurotensin receptor 1 (NTR1) with a specific antagonist, SR48692, could sensitize prostate cancer to ionizing radiation, thus improving outcomes of radiotherapy. We demonstrated that SR48692 selectively sensitizes prostate cancer cells but not normal prostate epithelial cells, most plausibly due to the differences in NTR1 expression. We also observed drug-dependent radiosensitization in orthotopic xenografts of human prostate cancer cells in mice. Importantly, the sensitization did not depend on AR expression, suggesting that anti-NTR1 treatment could be used to enhance radiotherapy of both androgen-dependent and independent tumors.

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Table of Contents

Final Report for the Project: W81XWH-08-1-0114

Project: Mechanisms of Radiosensitization by the Neurotensin Receptor Antagonist SR48692 in Prostate Cancer Models

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INTRODUCTION

Prostate cancer is the most common cancer in men and the second leading cause of cancer deaths in the United States (1,2). Radiotherapy is one of the standard treatment modalities for treatment (3), however, a major obstacle to effective radiotherapy is the limited radiation dose that can be safely delivered to the prostate (less than 85 Gy) (4,5). Unfortunately, at this dose level a significant proportion of tumors are resistant, either not responding or recurring after treatment. In a combined treatment, a long-term androgen depletion therapy (ADT, 2-3 years) improved the outcome of radiotherapy; however, a long-term hormone treatment has been shown to have significant side effects (6), and the side effects of even a short-term androgen suppression may also be substantial. In addition, recurrent tumors may become androgen-independent and resistant to ADT. An alternative to radiation dose increase would be to use radiosensitizing agents selectively targeting cancer cells while sparing normal tissue, thus minimizing radiation toxicity by lowering effective therapeutic doses.

Several different factors could participate in prostate cancer development, progression, and resistance to antitumor therapy. One of such possible mechanisms involves intra-prostate neuroendocrine cells and their secretions, which can aid cancer cell proliferation and survival. Neuroendocrine (NE) cells exist in the normal prostate gland, regulating prostatic growth, differentiation and secretion. However, clusters of NE-like cells are also found in most prostate tumors, and the presence of extensive NE features in tumors is an indication of increased aggressiveness and androgen independence (7-9). These NE-like cells often arise from cancer cells through the process of neuroendocrine trans-differentiation (8,10). The NE-like cells secrete a variety of factors, including parathyroid hormone-related peptides, serotonin, calcitonin, bombesin-related peptide, and neurotensin, that enhance DNA synthesis, proliferation and migration of cancer cells *in vitro* and i*n vivo*.

One of the neuropeptides secreted by NE-like prostate cells is neurotensin (NT)(11), a 13 amino acid peptide that has numerous physiologic effects (12) mediated predominantly through its cognate high-affinity receptor, neurotensin receptor 1 (NTR1). Stimulated NTR1 activates multiple pathways, namely mobilization of intracellular Ca^{2+}, production of cyclic AMP and GMP, and formation of inositol triphosphate, resulting in important physiological responses in both the central nervous system and periphery. However, we and others have shown that NTR1 is aberrantly expressed and activated in aggressive prostate cancer cells (13-15), and stimulation with NT increased MAP and PI3 kinase activation (16) and EGFR, Src and STAT5 phosphorylation (13,16), resulting in enhanced DNA synthesis, cell proliferation and survival. NTR1 signaling may not only be responsible for increased proliferation but for the intrinsic radioresistance of cancer cells. Thus, we hypothesized that inhibition of the NTR1 receptor and its downstream signaling represents a viable target to enhance the sensitivity of prostate cancer to radiotherapy.

BODY

1) SR48692 sensitizes PC-3M prostate cancer cells to ionizing radiation *in vitro* (SOW Task 1)

NTR1 can be selectively and efficiently inhibited by the commercially available small molecule antagonist, SR48692 (Meclinertant, Sanofi-Aventis) (17-19). We tested the hypothesis that inhibiting the NTR1 receptor, and therefore its downstream pro-proliferation and pro-survival signals, will enhance cell killing effects of ionizing radiation in prostate cancer cells.

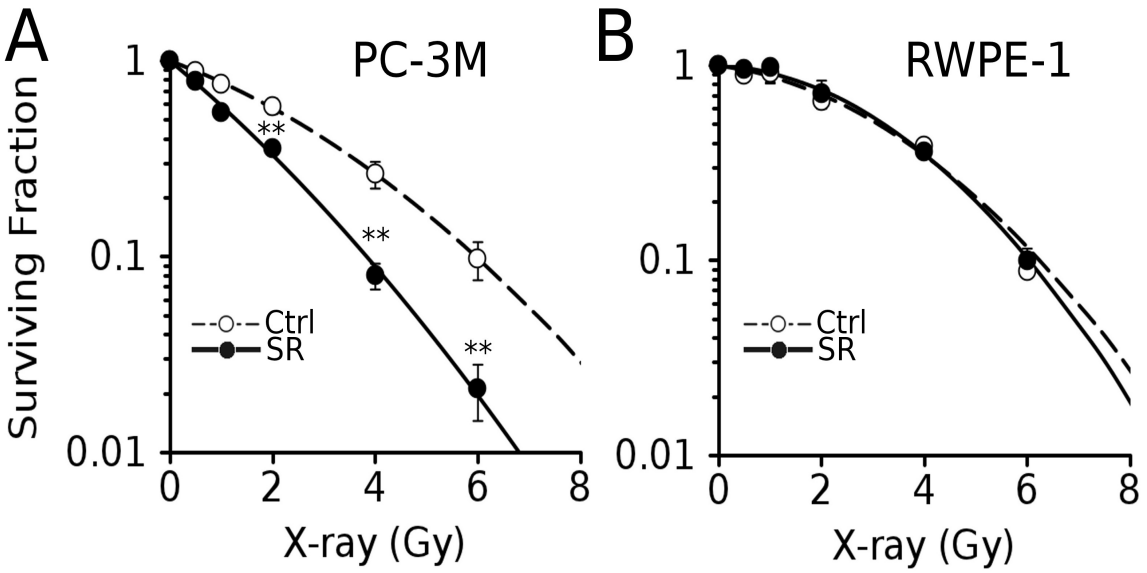

Figure 1. Blocking NTR1 receptor sensitizes prostate cancer cells to ionizing radiation. (A) Clonogenic survival of PC-3M cells treated with SR48692 and radiation. Cells were incubated with 1 μM SR48692 for 24h (SR) or left untreated (Ctrl), irradiated and re-plated for colony formation. Results are normalized for effects of the drug alone and fitted to a standard linear-quadratic model. Data points are derived from at least four independent experiments (±SEM); *, ** denote statistical significance at $p < 0.05$ and <0.01, respectively, compared to radiation only. **(B)** Clonogenic survival of RWPE-1 normal prostate epithelial cells treated with SR48692 and radiation.

For initial experiments, we chose an androgen-independent and highly metastatic prostate adenocarcinoma cell line (PC-3M) and a non-tumorigenic prostate epithelial cell line (RWPE-1). Cells were treated with an NTR1 antagonist, SR48692, at 1 μM for 24h, irradiated and their colony forming (clonogenic) ability assessed. At this concentration, SR48692 significantly ($p<0.01$) enhanced radiation effects in PC-3M cells (Fig. 1A), resulting in a decrease in surviving fraction at 2 Gy (SF_2) from 0.575 to 0.331, and a dose enhancement ratio at 37% survival (DER_{37}) of 1.77. The combined treatment also resulted in narrowing of the survival curve shoulder, which reflects the decrease in cellular capacity for sublethal damage repair, stemming from either diminished DNA damage signaling, damage repair capabilities or triggering of apoptosis. Importantly, SR48692 pretreatment did not sensitize normal epithelial RWPE-1 cells (Fig. 1B). As demonstrated in the graph, at the clinically relevant dose of 2 Gy, the difference between surviving fractions (SF2) in RWPE-1 and PC-3M cells treated with SR48692 and irradiated is approximately 2.1. Therapeutic gain of this magnitude has high clinical significance for radiotherapy of prostate cancer.

The difference between these two cell lines can be explained by our hypothesis that only cells expressing NTR1 will respond to SR48692 treatment. We confirmed that neurotensin receptors (NTR1, NTR2 and NTR3), are differentially expressed between normal and cancer prostate cell lines. Figure 2A shows that NTR1 protein, the specific target of SR48692, is expressed in PC-3M cells but not in RWPE-1 cells. Surprisingly, the messenger RNA for NTR1 was present in both cell lines (Fig. 2B), although the level in RWPE-1 was ~50% lower than in PC-3M cells (Fig. 2C). Both cell lines contained similar levels of mRNA for NTR3 (Fig. 2B, NTR3 lanes), but none for NTR2 (Fig.

2B, NTR2 lanes). Protein levels of NTR3 and NTR2 correlated with mRNA levels (data not shown). In addition, PC-3M expressed mRNA for the neurotensin/ neuromedin gene, while the neurotensin-specific RT-PCR product was absent in RWPE-1 reactions (Fig. 2B, NT lanes). These observations raise the possibility of autocrine stimulation of prostate cancer cells through endogenously expressed agonist (NT) stimulating expressed NTR1. Since there is evidence that majority of human prostate tumors express NTR1, while normal surrounding tissues do not, SR48692 could become a very important and clinically beneficial radiosensitizer for prostate radiotherapy.

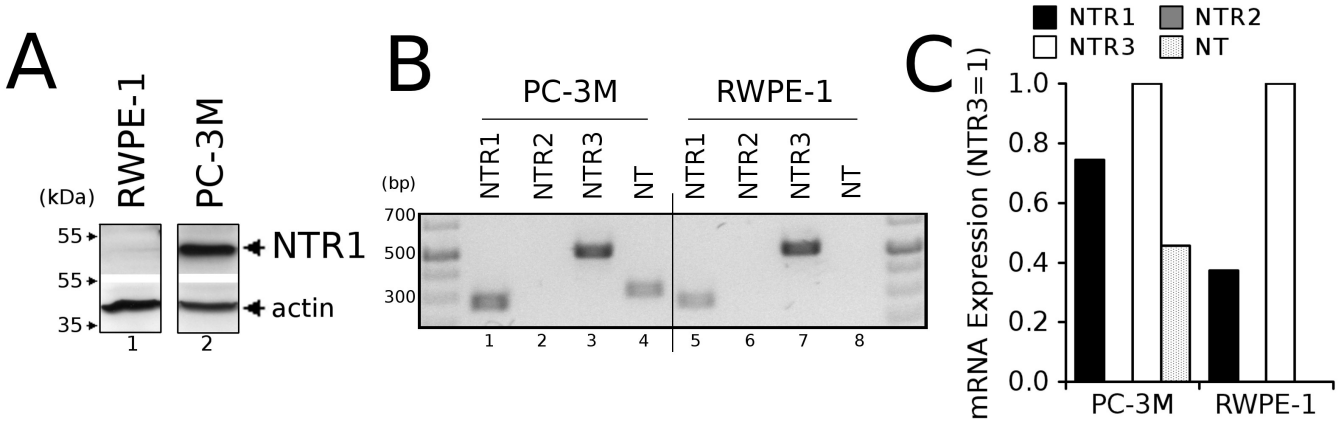

Figure 2. Neurotensin receptors are differentially expressed in normal and cancer prostate cells. (A) Expression of NTR1 receptor protein in RWPE-1 and PC-3M cell lines assessed by Western blotting. **(B)** Expression of neurotensin receptors (NTR1, 2, 3) and neurotensin (NT) mRNA in RWPE-1 and PC-3M cell lines assessed by semi-quantitative RT-PCR. **C)** Quantification of RT-PCR products from agarose gel electrophoresis (Panel B). Expression of NTR3 was used to normalize signal intensities.

As demonstrated in Figure 3, SR48692-induced radiosensitization is dose- and time-dependent, reaching a maximum at 1 μM and 24h of treatment.

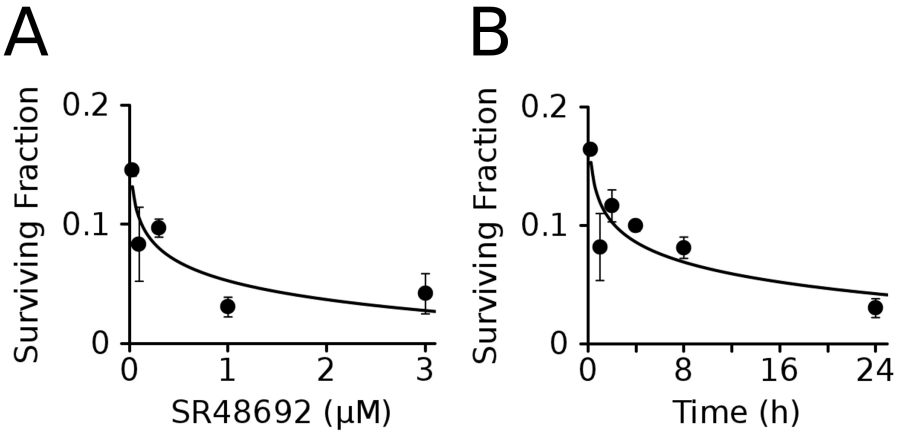

Figure 3. SR48692 radiosensitizes PC-3M cells in a dose- and time-dependent manner. (A) Cells were incubated with SR48692 (0-3 μM) for 24h, or **(B)** with 1 μM SR48692 for 1 to 24h, irradiated with 6 Gy X-rays and subjected to the colony formation assays.

2) SR48692 sensitizes PC-3M prostate cancer cells xenografts to radiotherapy *in vivo* (SOW Task 2)

The orthotopic (intra-prostate inoculation) human prostate cancer model was used to test the hypothesis that SR48692 can be used as a radiosensitizing agent *in vivo*. The xenografts were established from PC-3M-luc cells, a metastatic variant of human prostate adenocarcinoma PC-3 cells, that were engineered to stably express luciferase. This system allows for non-invasive continuous measurements of cancer volume/size, even for xenografts located inside the body. In addition, our preliminary experiments demonstrated that in this system bioluminescence intensity correlates very well with tumor size as measured by calipers. The schematic outlines of the *in vivo* experiments are presented in Fig. 4A and the results in Fig. 4B and 4C.

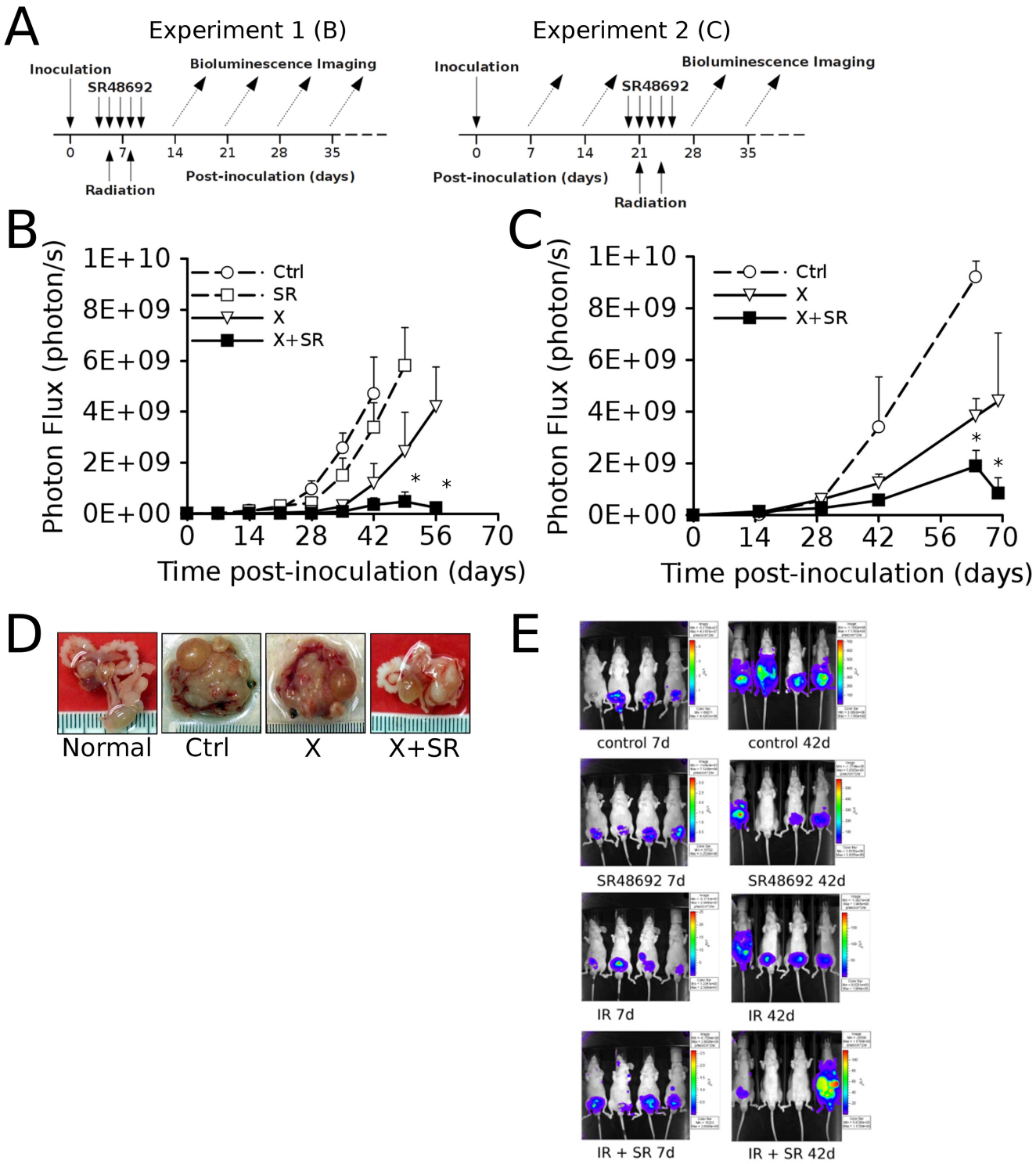

Figure 4. Effects of NTR1 antagonist combined with radiation on the growth of PC-3M orthotopic xenografts in nude mice. (A) Schematic description of Experiments 1 and 2. **(B)** Experiment 1 – five days post-inoculation mice were randomized, treated with SR48692 (SR, 25 mg/kg) for five consecutive days and irradiated (X, 2.5 Gy delivered to prostate area only) on second and fourth day of treatment. Bioluminescence imaging was performed on a weekly basis. Data points represent means from 8 animals in the group (±SEM); *, $p < 0.05$ compared to radiation only. **(C)** Experiment 2 – the presence and size of tumors were assessed two weeks post-inoculation, and the mice were randomized into four treatment groups. Treatment was performed during week 4 as described for panel A. **(D)** Representative images from C) of the urogenital system in non-treated (normal), PC-3M inoculated (Ctrl) and treated (X and X+SR) mice euthanized 50 to 70 days post-grafting. **(E)** Representative bioluminescence images from A).

In the first experiment, animals (8 per treatment group) were randomized on day 5 post-inoculation, and drug treatment / irradiation was started immediately thereafter. In the second experiment, tumor development was monitored by bioluminscence for 14-21 days, then the animals with similar tumor burden were randomized into treatment groups and treated. Treatment regimen was the same in both experiments and consisted of five daily does of SR48692 (25 mg/kg) followed by 2.5 Gy IR on days 3 and 5 (Fig. 4A). Figure 4B and C show the tumor burden (measured as bioluminescence from PC-3M cells, Fig. 4E) in animals in experiments 1 and 2, respectively. In both experiments, SR48692 alone (SR) had no significant effect on tumor progression, while radiation alone (X) was only partially efficacious. However, the combined treatment (X+SR) showed the most prominent effect, significantly reducing tumor growth in the treated animals (X vs. X+SR, $p<0.05$). This reduction was especially noticeable when the combined treatment was used on animals inoculated only five days before (experiment 1, Fig. 4B). The reduced tumor burden was confirmed by visual examination of excised urogenital tracts (Fig. 4D, representative samples from experiment 2).

3) SR48692 is relatively non toxic as a single agent treatment (SOW Task 1, 2 and 4)

In the following experiments, we have tested the possibility that SR48692 alone is cytotoxic to prostate cancer cells. PC-3M cells were treated with the drug at doses ranging from 0 to 10 μM for 24h, and then counted directly for short-term growth inhibition assessment. For long-term clonogenic survival, the cells were plated at 100 cells per dish, treated continuously for 7-10 days, and the surviving colonies were stained and scored. In addition, the drug effects on cell cycle progression were determined. As shown in Fig 5, SR48692 alone does not modify cell cycle distribution and is relatively non-toxic to PC-3M cancer cells.

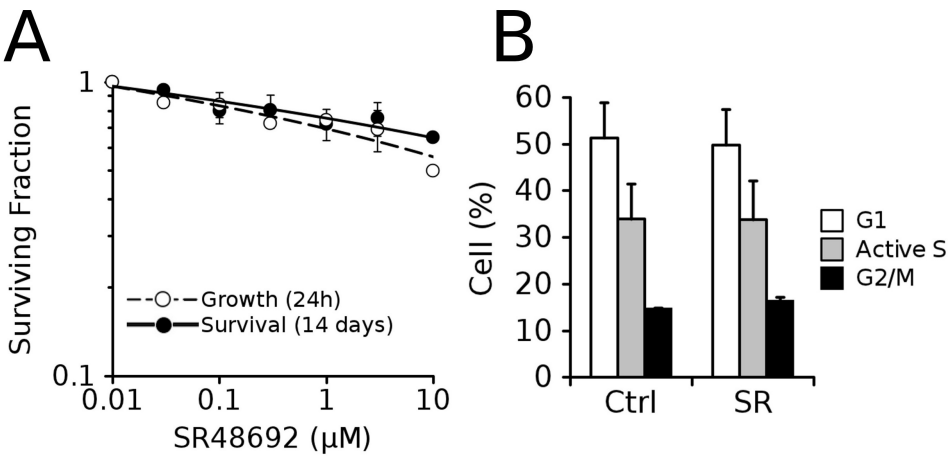

Figure 5. Effects of SR48692 on the growth and survival (A) and cell cycle distribution (B) of PC-3M prostate cancer cells. (A) Cells were treated for 24h and counted (Growth) or treated continuously for 14 days and colonies were counted (Survival). **(B)** Cells were treated with 1μM SR48692 for 24h and the cell cycle distribution determined using BrdU incorporation (for active S phase) and 7AAD staining (for DNA content).

4) SR48692 sensitizes prostate cancer cells to ionizing radiation independently of their androgen receptor dependence (SOW Task 1 and 4).

To determine if SR48692 radiosensitizing activity is confined only to androgen-insensitive PC-3M cells or also is present in androgen-sensitive prostate cancer cells, we tested LNCaP and its derivative, C4-2B, in clonogenic survival assays. Both cell lines express androgen receptor and respond to androgen stimulation, unlike PC-3M, which is AR-negative and androgen-insensitive. The difference between the two cell lines is that C4-2B can grow in the absence of androgen, while LNCaP growth is androgen-dependent. In addition to androgen receptor status, LNCaP and C4-2B cell lines also differ significantly from PC-3M by their p53 status: both express wild-type protein, while PC-3M cells are p53-null. As demonstrated in Figure 6A and B, both cell lines are sensitized to radiation by pretreatment with SR48692 (1 μM/24h). The decreases in SF_2 (LNCaP from 0.599 to 0.419, C4-2B from 0.481 to 0.336) following combined treatment are statistically significant ($p<0.05$), although smaller in magnitude than the decrease in SF_2 in PC-3M cells. The DER_{37} calculated for

both cell lines equals 1.43, also smaller than the DER_{37} established for PC-3M cells. Based on these observations, we hypothesize that inhibition of NTR1 radiosensitizes prostate cancer cells independently of their AR and p53 status.

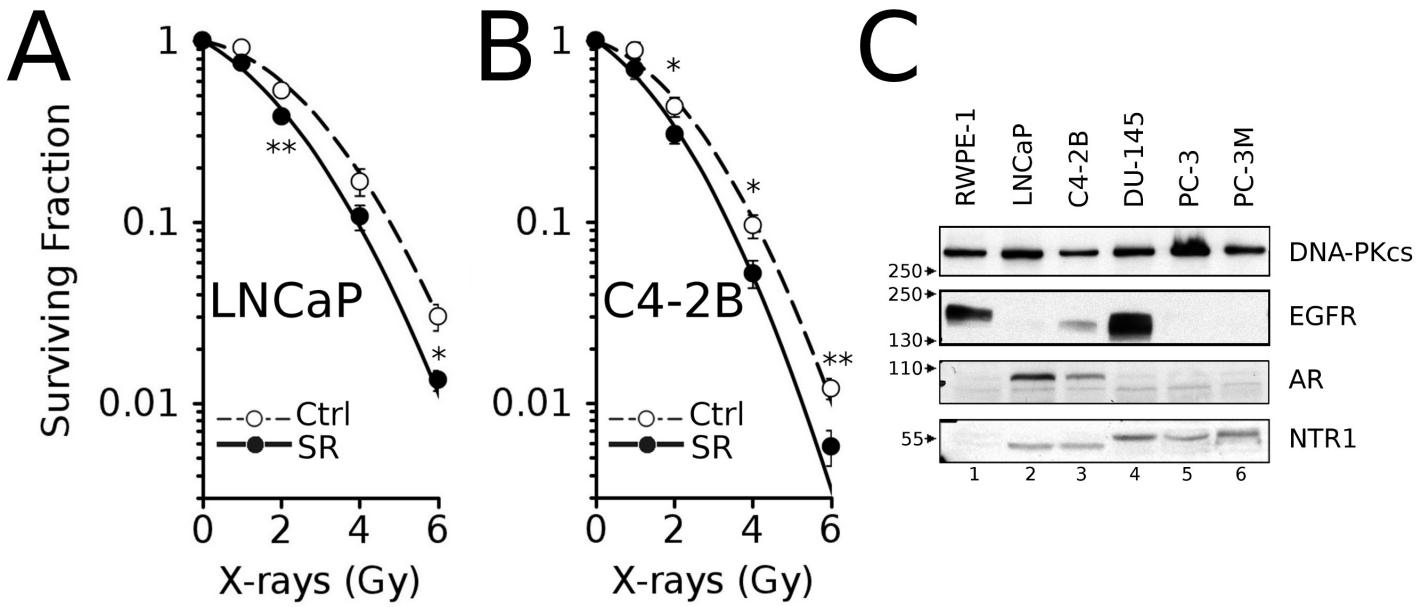

Figure 6. Inhibition of NTR1 radiosensitizes androgen receptor expressing and androgen-sensitive prostate cancer cells. LNCaP **(A)** or C4-2B **(B)** Prostate cancer cells were pre-treated with SR48692 (SR, 1μM for 24h) or sham-treated (Ctrl), irradiated and subjected to a colony formation assay. Data were gathered from at least three independent experiments (±SEM); * and ** denote statistical significance at $p < 0.05$ and <0.01, respectively, compared to radiation only. **(C)** Expression levels of several protein markers in different prostate cell lines.

5) SR48692 inhibits neurotensin-induced androgen receptor phosphorylation and stabilization (SOW Task 1 and 4).

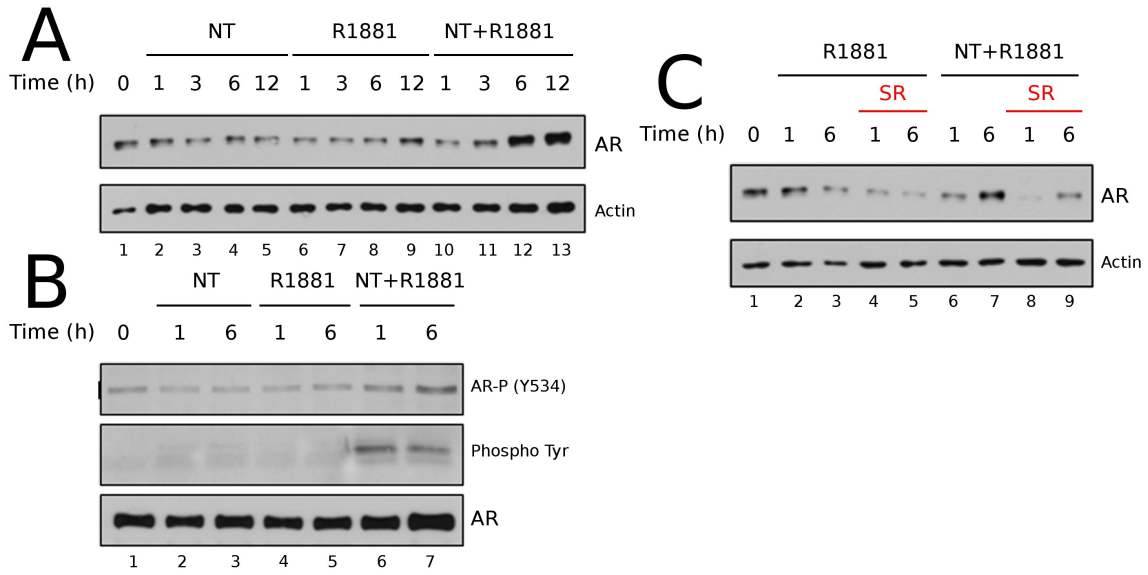

Figure 7. Neurotensin/NTR1 signaling enhances AR (Y534) phosphorylation and stabilization of AR protein. (A) NT treatment (100nM) stabilizes AR protein. **(B)** NT treatment enhances androgen-induced AR phosphorylation (Y534). **(C)** SR48692 (1μM/24h) inhibits NT-induced AR phosphorylation and stabilization.

Androgen ablation combined with radiotherapy is a key strategy for the treatment of patients with locally advanced, high-risk prostate cancer. Although systemic ADT causes initial suppression of prostate tumor growth, cancer cells continue to be stimulated by androgen produced by the adrenal gland or within the tumor itself (autocrine stimulation). The relationship between NT, androgen, and their respective receptors is complex. Androgen deprivation is associated with NE-like differentiation of cancer cells and an increase in NT expression (20). Thus, in the absence of or at very low levels of androgen, NT activates critical pro-survival signaling pathways which could

result in enhanced resistance to radiotherapy. We have discovered that NT treatment enhances AR stabilization (Fig. 7A) and phosphorylation (Fig. 7B) in LNCaP cells (AR-positive and androgen-dependent), and this effect can be abrogated by treatment with SR48692 (Fig. 7C). LNCaP cells were serum-starved and subsequently stimulated for the indicated times with NT (100 nM), synthetic androgen R1881 (0.1nM), or a combination of the two, and the lysates were analyzed by immunoblotting and/or immunoprecipitation. This observation is extremely significant, since it demonstrates that NTR1 can mediate AR activation even in the absence of androgen, thereby promoting androgen-independent growth. Using an NTR1 antagonist could benefit patients not only by radiosensitizing cancer cells, but also by reducing androgen-independent growth.

6) Pretreatment with SR48692 enhances radiation-induced apoptosis in PC-3M cells (SOW Task 1)

Treatment of PC-3M cells with a combination of SR48692 (1 μM/24h) and radiation (6 Gy), resulted in a significant increase in apoptosis within 48 hours, as shown by the enhanced cleavage of PARP and phospho-S2065 DNA-PK (Fig. 8A, lower bands). Both proteins are known to be targets for caspase 3-mediated degradation during apoptosis (21,22). Consistent with this observation, there was a statistically significant ($p<0.05$) increase in caspase 3 and 7 activity, as measured with a specific fluorescent substrate in cells treated with combined agents (Fig. 8B, X+SR) when compared to radiation only treatment (Fig. 8B, X). It is worth to note that, at the dose and times assessed, the drug alone in the presence of serum did not induce cellular apoptotic death, in line with our previous results on the minimal effects of SR48692 on cell growth and cell cycle (Fig. 5).

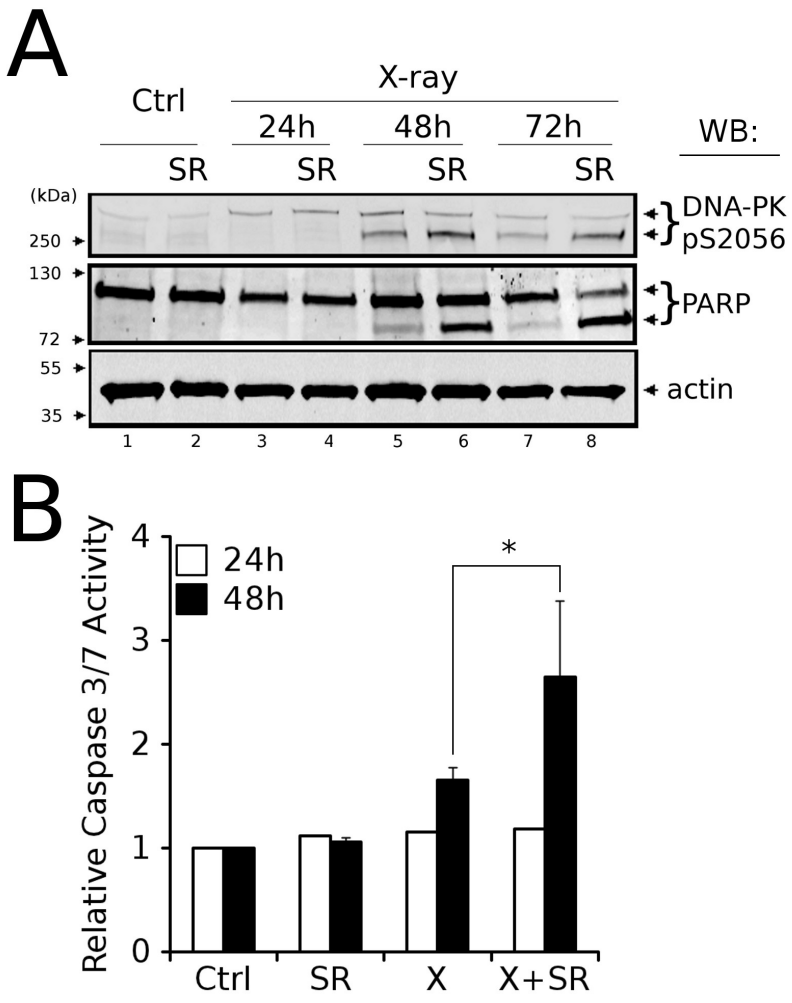

Figure 8. SR48692 treatment enhances apoptosis in irradiated prostate cancer cells. (A) PC-3M cells were treated with SR48692 (SR, 1 μM for 24h) and irradiated (X, 6 Gy), and samples including floating cells were collected at specified times (24, 48 and 72h post-irradiation). A representative Western blot shows full size and cleaved PARP and phosphorylated (pS2056) DNA-PK after indicated treatments. ß-actin was used as a loading control. **(B)** Caspase 3/7 activity in SR48692-treated (SR, 1 μM for 24h) and irradiated (X, 6 Gy) PC-3M cells was measured 24 and 48 hours post-irradiation as described in Materials and Methods. Fluorescence signals were normalized to the fluorescence of sham-treated controls (Ctrl). The results were obtained in two independent experiments (6-12 intra-experimental replicates), * indicate $p<0.05$.

7) Role of EGFR receptor signaling in radiosensitization induced by SR48692 in prostate cancer cell (SOW Task 1 and 4).

Amorino et al. (2007) (23) demonstrated that NT treatment induces Src-dependent EGFR phosphorylation and activation in PC-3M cells and that this activation can lead to enhanced growth rate and survival of cancer cells. Here, we confirmed that observation and demonstrated that SR48692 can completely block this stimulation, but has no effect on normal RWPE-1 cells (Fig. 9).

It is known that radiation induces EGFR phosphorylation and activates downstream signaling pathways leading to increased cell survival (24,25). Here, we determined if SR48692 pretreatment interferes with radiation- and/or NT-induced EGFR phosphorylation. For short-term studies in complete medium, the drug-treated (1 μM for 24h) PC-3M cells were irradiated (6 Gy), incubated for 5 min at 37 ºC, and lysed in CHAPS buffer. Total cellular EGFR was immunoprecipitated, and Western blotting was performed using antibodies against EGFR phospho-tyrosine 992 (pY992) and total phospho-tyrosine (pY20). Figure 10A shows that under these conditions, radiation induced an increase in EGFR phosphorylation as determined by both pY20 and pY992 phospho-specific antibodies. SR48692 alone did not affect EGFR phosphorylation levels; however, it reduced radiation-induced phosphorylation to 83% (pY992) and 67% (pY20) of initial levels.

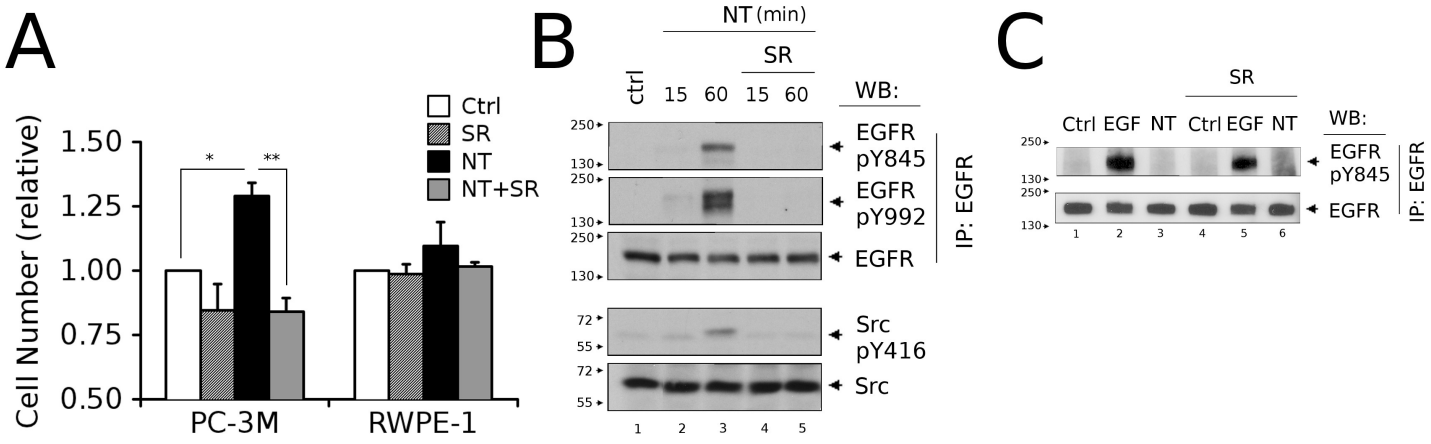

Figure 9. SR48692, NTR1 antagonist, inhibits neurotensin-induced EGFR phosphorylation/activation in prostate cancer cells. (A) SR48692 inhibits stimulatory effects of neurotensin in prostate cancer cells. PC-3M and RWPE-1 cells were serum-starved for 48h, pretreated with SR48692 (SR, 1 μM for 24h), stimulated with neurotensin (NT, 100 nM for 24h) and cell growth quantified. Results are normalized to the untreated control, and data points are from 2-4 independent experiments (±SEM). **(B)** NT-induced EGFR and Src phosphorylation in serum-starved PC-3M cells. Cells were lysed following 15 to 60 min incubation with NT (100 nM) +/- SR48692 (1 μM), cellular EGFR was immunoprecipitated, and EGFR phosphorylation was assessed using phospho-specific antibodies. Src phosphorylation (pY416) was assessed using straight Western blotting. **(C)** Under similar conditions (serum-free medium) NT (100 nM/1h) does not induce EGFR phosphorylation in RWPE-1 cells lacking NTR1 receptor. EGF stimulation (100 ng/mL for 5 min) was used as a positive control.

Figure 10B shows significant increase in the phosphorylation levels of EGFR (pY845 and pY992) and Src (pY416) in serum-starved PC-3M cells following an extended time course (15-60 min) of irradiation with 6 Gy alone (X) or combined treatment of radiation plus neurotensin (X+NT). The radiation-induced phosphorylation, although observed 5 min post-irradiation in Fig. 10A, is reduced to almost basal levels at 15 or 60 min following irradiation (Fig. 10B, X). With combined treatment (Fig. 10B, X+NT), EGFR and Src phosphorylation reaches a maximum at 60 min, closely following the kinetics of phosphorylation induced by NT alone (Fig. 9B). In both cases, pretreatment with SR48692 completely abrogate this long-term EGFR or Src phosphorylation (Fig. 10B, SR lanes).

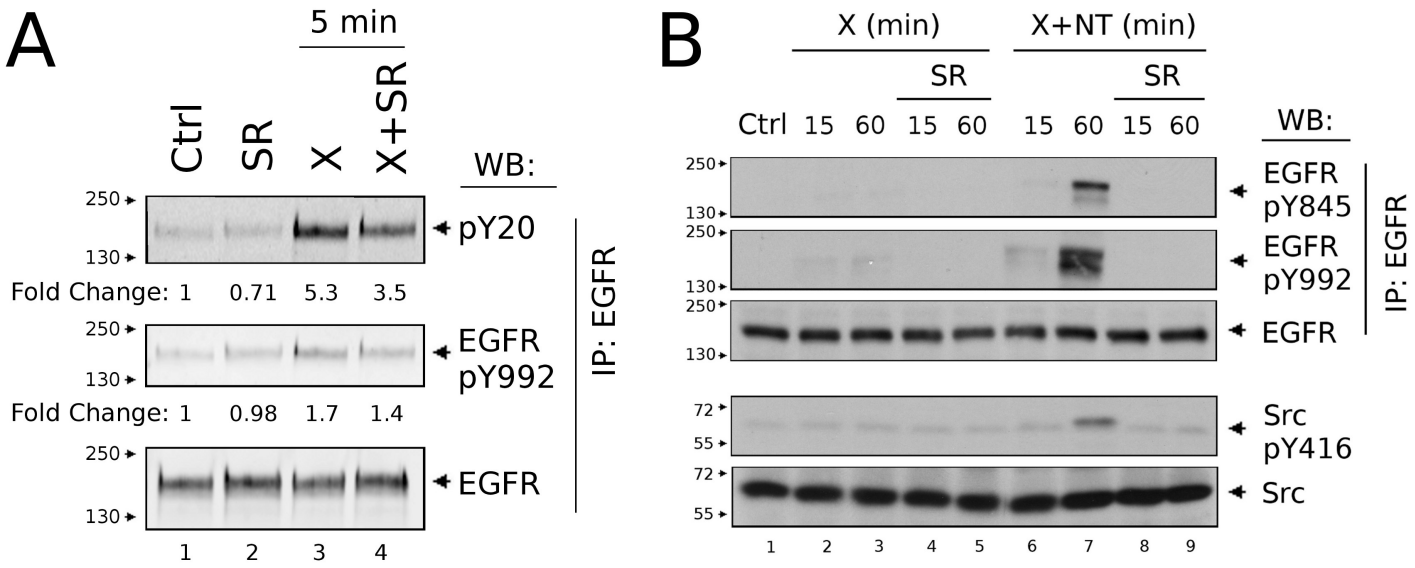

Figure 10. SR48692 inhibits ionizing radiation-induced EGFR phosphorylation/activation in prostate cancer cells. (A) PC-3M prostate cancer cells were treated with SR48692 (SR, 1 μM for 24h) and/or irradiation (X, 6 Gy), and cellular lysates were prepared after 5 min. Total tyrosine (pY20) and EGFR tyrosine 992 specific (pY992) phosphorylation were assessed in EGFR immunoprecipitates. Fold-changes represent the mean of two independent experiments, with phospho-specific signal intensities normalized to total EGFR levels. **(B)** PC-3M cells were pre-incubated with SR48692 (SR, 1 μM/24h), stimulated with neurotensin (NT, 100 nM/1h) as indicated and irradiated (X, 6 Gy). EGFR phosphorylation (Y845 and Y992), Src phosphorylation (Y416), and the expression of total EGFR and Src proteins were analyzed by immunopreciptiation and Western blotting.

The EGFR pathway is emerging as a promising target for tumor radiotherapy (26-28). The FDA has granted approval for the use of cetuximab (C225, Erbitux; ImClone), a monoclonal antibody directed to the EGFR, in combination with radiation for the treatment of advanced squamous cell carcinoma of the head and neck but limits its use only to patients overexpressing EGFR. Unfortunately, detectable EGFR expression was found only in 18% of prostate tumors in an extensive IHC study employing ~2,500 patient samples (29). In line with this observation, phase II trials of EGFR tyrosine kinase inhibitors alone, or in combination with ADT, failed to demonstrate significant clinical benefit (30,31). Our demonstration that targeting the trans-activation of the EGFR through the use of SR48692 suggests that this strategy might be effective in a subset of patients whose tumors express low, rather than high, EGFR.

KEY RESEARCH ACCOMPLISHMENTS

- We demonstrated that NTR1 antagonist SR48692 sensitizes human prostate cancer cells to ionizing radiation *in vitro*. Treatment with SR48692 enhances radiation-induced apoptosis and reduces cell survival.
- We showed that SR48692 significantly improves the outcomes of prostate cancer xenografts radiotherapy *in vivo*.
- We demonstrated that SR48692 does not sensitize human prostate normal epithelial cells to ionizing radiation *in vitro* (the therapeutic gain of about 2). We postulate that the radiosensitizing activity of SR48692 depends on the expression of NTR1 receptor in prostate cells. This establishes the foundation of cancer specificity for SR48692 radiosensitizing activity.
- We demonstrated that radiosensitizing activity of SR48692 is not dependent on androgen receptor (AR). Moreover, SR48692 blocks neurotensin-induced AR phosphorylation/ stabilization. Therefore, blocking NTR1 receptor could provide additional benefits to androgen-depletion therapy.
- We demonstrated that the radiosensitizing activity of SR48692 involves disruption of the EGFR receptor signaling cascade and is affected by the EGFR receptor levels in prostate cells.

REPORTABLE OUTCOMES

The first manuscript covering majority of the results from this project has been submitted to Cancer Research in May 2011 [M1]. A second manuscript describing our findings on the role of androgen receptor and androgen-dependence in cellular responses to SR48692 will be submitted in the near future [M2].

In addition, the results obtained during three years of this project were presented in the form of posters or oral presentations at several scientific meetings [P1-P5], most recently at the Annual Meeting of the American Association for Cancer Research [P6] and Department of Defense IMPACT 2011 Meeting [P7]. A presentation concluding our discoveries was accepted for this year's International Congress on Radiation Research [P8] meeting.

Nnew insight into the role(s) of NTR1 in prostate carcinogenesis, as well as the role(s) of neuroendocrine cells in cancer progression and resistance to radiation gained in this project were the basis for a new grant application: “Effects of prolonged exposure to space radiation on carcinogenesis and neuroendocrine differentiation in human prostate models”, submitted to the NASA Human Research Program and funded for 3 years [G1]. An additional grant application based on, and further developing this project, has been recently submitted to NIH [G2]. The preliminary scores suggest that the application could be funded in 2012.

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Manuscripts:

[M1] Valerie NCK, Casarez EV, DaSilva JO, Dunlap-Brown M, Parsons SJ, Amorino GP & Dziegielewski J. Inhibition of neurotensin receptor 1 selectively sensitizes prostate cancer to ionizing radiation in vitro and in vivo. *Cancer Res., 2011, submitted/under review*.

[M2] Casarez EV, Valerie NCK, DaSilva JO, Larner JM, Parsons SJ & Dziegielewski J. The role of neurotensin receptor 1 in prostate cancer androgen-independent growth and resistance to radiotherapy. *2011, in preparation*.

Presentations:

[P1] Amorino G, Pemberton B, Dunlap-Brown M, & Parsons SJ. Neurotensin receptor antagonist SR48692: potential radiosensitizer in mice. *100th Annual Meeting of the American Association for Cancer Research, Denver, CO, 2008*.

[P2] Dziegielewski J, Pemberton BL, Dunlap-Brown ME, Larner JM, Parsons SJ & Amorino GP. SR48692, a specific neurotensin receptor (NTR1) antagonist, sensitizes prostate cancer cells to ionizing radiation, in both in vitro and in vivo models. *55th Annual Meeting Radiation Research Society, Savannah, GA, 2009*.

[P3] Dziegielewski J, Pemberton BL, Dunlap-Brown ME, Parsons SJ, Amorino GP & Larner JM. The specific neurotensin receptor (NTR1) antagonist, SR48692, sensitizes prostate cancer cells to ionizing radiation, in both in vitro and in vivo models. *51st ASTRO Annual Meeting, Chicago, IL, 2009*.

[P4] Dziegielewski J, Valerie NCK, Casarez EV Jr., Dunlap-Brown ME, Hosing AS, Parsons SJ & Larner JM. Targeting neurotensin receptor 1 (NTR1) signaling pathway(s) sensitizes prostate cancer cells to ionizing radiation. *Gordon Research Conference – Radiation Oncology, Galveston, TX, 2010.*

[P5] Dziegielewski J, Valerie NCK, Dasilva JO, Hosing AS, Parsons SJ & Larner JM. Interplay between neurotensin receptor 1 (NTR1) and epidermal growth factor receptor (EGFR) in responses

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[P7] Dziegielewski J, Valerie NCK, Dasilva JO, Casarez EV Jr., Anaganti S, Dunlap-Brown ME, Parsons SJ, Larner JM & Amorino GP. Mechanisms of radiosensitization by the neurotensin receptor antagonist SR48692 in prostate cancer models. *Department of Defense IMPACT 2011 Meeting, Orlando, FL, 2011*.

[P8] Valerie NCK, Casarez EV Jr, Parsons SJ, Larner JM & Dziegielewski J. Neurotensin receptor 1 (NTR1), a novel target for tumor radiosensitization. *14th International Congress on Radiation Research, Warsaw, Poland, 2011*.

Grant applications:

[G1] Dziegielewski J (PI). Effects of prolonged exposure to space radiation on carcinogenesis and neuroendocrine differentiation in human prostate models. *NASA Ground-Based Studies in Space Radiobiology Award NNX10AC13G (2010-2013)*.

[G2] Dziegielewski J (PI). Molecular mechanism of NTR1 antagonist – induced radiosensitization in prostate cancers. *NIH R01 application 2011 (under review)*.

CONCLUSION

Based on the incidence of cancer recurrence and radioresistance, improvements in radiation therapy for prostate tumor treatments are urgently needed. Here, we have demonstrated that inhibition of the neurotensin receptor 1 (NTR1) is a novel and efficient method for radiosensitization of prostate cancer. Our results show that combined treatment of SR48692, a selective inhibitor of NTR1, and ionizing radiation efficiently kills cancer cells in vitro and significantly lowers tumor burden in vivo. Most importantly, the combined treatment provides selectivity between normal and cancer prostate cells. Additionally, the combination of NTR1 antagonist and radiotherapy is effective independent of androgen receptor and p53 status. Based on these observations, we strongly believe that NTR1 inhibition could improve the outcomes of radiotherapy in patients with both early (AR-dependent) and late (AR-independent) stages of prostate cancer. This work will also contribute to the development of modern personalized therapy for prostate tumors by establishing the relationship between NTR1 expression, neurotensin signaling, and tumor resistance to therapy. We will continue the work on the role of NTR1 in prostate cancer development, progression and resistance to therapy, with the ultimate goal of testing the idea in clinical trials and introducing it into everyday clinical practice.

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